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Keeping Autoimmunity in Check: How to Control a Th17 Cell Controller

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The transcription factor IRF-4 is necessary for Th17 cell differentiation. In this issue of *Immunity*, Chen et al. (2008) show that IRF-4-binding protein is a critical negative regulator of IRF-4 function, regulating production of the cytokines IL-21 and IL-17.

The discovery of T helper 17 (Th17) cells has markedly advanced our understanding on many inflammatory and autoimmune diseases (Dong, 2008). Elevated amounts of interleukin-17 (IL-17) and IL-21 are found in rheumatoid arthritis (RA), systemic lupus erythematosus, and psoriasis patients. In addition, blocking of IL-17 or IL-21 in a mouse model of RA decreases joint damage and destruction of cartilages and bones. Given the pathogenic function of Th17 cell cytokines in autoimmune disease such as RA, it is critical to understand the control of Th17 cell differentiation. However, the molecular mechanisms involved in the negative regulation of Th17 cell differentiation have not been fully defined. In this issue of *Immunity*, Chen et al. describe IRF-4-binding protein (IBP) as a key factor in controlling T cell-mediated autoimmunity (Chen et al., 2008).

Th17 cells produce IL-17, IL-17F, and IL-22, which together regulate inflammatory responses by tissue cells (Dong, 2008). Th17 cell differentiation in mouse is initiated by the cytokines TGF-β and IL-6. In addition, Th17 cells also highly express IL-21, an autocrine cytokine that synergizes with TGF-β to induce Th17 cell differentiation (Dong, 2008). STAT3, a transcription factor downstream of IL-6 signaling, is essential for Th17 cell differentiation, possibly via the induction of two nuclear receptors, RORα and RORγt. Interestingly, IL-21 expression requires STAT3 but not RORγ (Nurieva et al., 2007). Another molecule, interferon regulatory factor 4 (IRF-4), is also critical for the generation of Th17 cells, and consequently for the development of experimental autoimmune encephalomyelitis (EAE), a mouse model of multiple sclerosis (Brustle et al., 2007). IRF-4-deficient Th cells fail to differentiate into Th17 cells. In addition, *Irf4*$^{-/-}$ Th cells exhibit impaired expression of RORγt (Brustle et al., 2007).

Gupta et al. (2003) identified IRF-4-binding protein (IBP), which exhibits marked homology with the protein SWAP-70. In contrast to SWAP-70, IBP is highly expressed in T cells and, upon T cell receptor (TCR) engagement, is rapidly phosphorylated by the kinase Lck and recruited to the immunological synapse (Gupta et al., 2003). The same group reported that IBP-deficient mice on C57BL/6 and 129 mixed background spontaneously developed a lupus-like syndrome, including the accumulation of effector and memory T cells and IgG^{+} B cells, profound hypergammaglobulinemia, and autoantibody production (Fanzo et al., 2006). Thus, it was proposed that IBP is required for optimal T cell effector function, lymphocyte homeostasis, and the prevention of systemic autoimmunity.

To further determine the preventive role of IBP in the development of autoimmunity, Chen et al. (2008) backcrossed IBP-deficient mice on Balb/c background and then crossed to DO11.10 TCR transgenic mice. Chen et al. (2008) observed that at 7 weeks of age, these mice spontaneously developed arthritis characterized by joint erythema and swelling and increased titers of autoantibodies such as rheumatoid factor, collagen II antibodies, and cyclin citrullinated peptide (CCP) antibodies. Beginning at 3 months of age, these mice started to die, as a result of the development of large-vessel vasculitis. In exploring the mechanisms underlying these fatal disorders associated with IBP deficiency, Chen et al. (2008) first examined the possibility of defective central tolerance. However, using two models of central tolerance, they found that spontaneous development of arthritis and vasculaties in IBP-deficient DO11.10 mice was not due to failure of elimination of autoreactive T cells in the thymus by negative selection.

In addition, they did not detect any defect in development and function of regulatory T cells in these mice. In contrast, they found that IBP-deficient DO11.10 T cells were hyporesponsive to high antigenic doses but hyperresponsive to low doses. Analysis of 6-week-old IBP-deficient mice revealed a striking accumulation of KJ1-26^{hi} T cells displaying effector and memory phenotype (CD62^{lo}CD44^{hi}) with upregulated expression of CD69 and the costimulatory molecule inducible T cell costimulator (ICOS). Transfer of this KJ1-26^{hi} population of cells into nude Balb/c mice led to sudden death of recipients. Thus, the absence of IBP led to abnormal responsiveness and spontaneous activation in TCR transgenic cells with pathogenic effector phenotype. However, the underlying mechanisms have not been defined in the current study. Whether IRF-4 binding is required for the function of IBP in T cell activation should be addressed in the future.

Chen et al. (2008) also assessed the role of IBP in the control of pathogenic functions of effector T cells. They detected substantially elevated amounts of IL-17 and IL-21 produced by mutant cells compared to wild-type cells. Interestingly, enhanced amounts of both cytokines were detected in the absence of Th17 cell polarizing cytokines (in neutral condition). Considering that IL-21 not only is expressed by Th17 cells, but also regulates Th17 cell differentiation, abnormal production of IL-21 production by IBP-deficient CD4$^+$ T cells might drive them toward Th17 cell differentiation. This idea needs to be confirmed in later studies. In vivo, elevated amounts of these cytokines were detected in the sera and joints of arthritic IBP-deficient DO11.10 mice. Thus, early development of autoimmunity in IBP-deficient DO11.10 mice correlated with greatly enhanced production of IL-17 and IL-21.

Given that IBP was originally identified to interact with the transcriptional factor IRF-4 and that the latter was involved in Th17 cell differentiation, the major function of IBP might be to control IRF-4 transcriptional activity. Chen et al. (2008) performed genetic analysis and found that enhanced IL-21 and IL-17 production in IBP mutant cells was abrogated on an IRF-4-deficient background. Importantly, the authors showed that IL-21 expression in wild-type cells requires IRF-4. Thus, IRF-4 appears to be more important than RORγ in IL-21 expression. The relationship of IRF-4 and STAT3 would need to be examined in the future. Moreover, Chen et al. (2008) reported that IRF-4 could act as a transactivator of the IL-21 gene promoter. Thus, absence of IBP leads to enhanced activation of the IL-21 gene transcription by IRF-4. The authors further present the data that IBP and IRF-4 can physically interact, and this binding is dependent on carboxyl terminus portion of IBP.

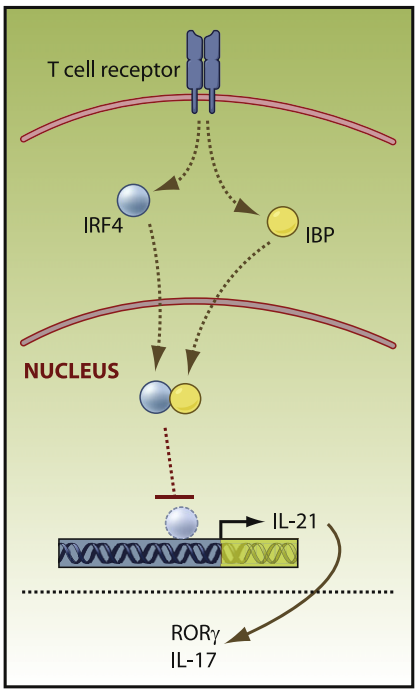

Figure 1. IBP Regulates the Ability of IRF-4 to Activate IL-21 Promoter

Evidence presented by Chen et al. (2008) demonstrated the specific interaction of IBP and IRF-4 in the nucleus. This interaction prevents the binding of IRF-4 to its target site in the IL-21 promoter. Absence of IBP leads to enhanced targeting of the IL-21 gene promoter by IRF-4, consequently leading to abnormal expression of RORγ and IL-17 and to the development of autoimmunity.

The current study conclusively demonstrates that IBP is a critical brake of autoimmune responses. It acts by two folds: one is to prevent hyperresponsiveness to low-affinity TCR engagement, and the other is to restrict the ability of IRF-4 in programming Th17 cell differentiation. However, additional studies need to be performed to advance our knowledge of this important regulator. First, what is the relationship of the above two functions of IBP in T cells? IBP shares substantial homology with SWAP-70, a Rac activator. It remains to be addressed whether IBP has any activity in regulating the Rac and Rho family of GTPases, which may consequently influence immunological synapse formation and activation of p38 and JNK MAP kinases. Whether this aspect of IBP function contributes to its regulation on TCR signaling and Th17 cell differentiation needs to be understood.

Second, what is the direct function of IBP in Th17 cell differentiation? The authors have shown IL-21 as a target of IRF-4 and IBP. Interestingly, the authors also found that IBP-deficient T cells exhibited elevated expression of ICOS in vivo. ICOS is required for the pathogenesis of an arthritis model and regulates IL-17 and IL-21 expression (Dong and Nurieva, 2003; Nurieva et al., 2008). Enhanced expression of ICOS in *san roque* mice has been shown to be associated with IL-21 expression and development of spontaneous lupus and arthritis (Vinuesa et al., 2005). It would be interesting to test whether the phenotypes of IBP mutant mice are dependent on ICOS. This may help understand the initial trigger of an ICOS-IL-21 circuit that is pathogenic for autoimmunity.

In addition to Th17 cells, IL-21 is also produced by follicular helper T (Tfh) cells and was recently shown to mediate the differentiation of these cells (Nurieva et al., 2008; Vogelzang et al., 2008). The abnormal B cell activation and secretion of pathogenic autoantibodies seen in IBP mutant mice may be caused by increased differentiation and function of Tfh cells. It is thus important to understand whether IRF-4 is required for IL-21 expression after Tfh differentiation and whether this regulation has any importance in pathogenesis of antibody-mediated autoimmune diseases. These studies will greatly enrich our understanding on Tfh cells, as another lineage of Th cells that help the humoral immunity.

In conclusion, the work described by Chen et al. (2008) provides exciting insights into the function of IBP as a negative regulator of TCR signaling to low doses of antigens and of IRF-4 function in regulation of proinflammatory cytokines (Figure 1). Further understanding of IBP function may shed light on the pathogenesis of human autoimmunity, as supported by the lethal phenotypes associated with IBP mutation.

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CD44 Keeps Tumor Killers Polarized

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In this issue of *Immunity*, Mrass et al. (2008) report that CD44 critically contributes to the stabilization of polarity in migrating cytotoxic T lymphocytes by anchoring cytoskeletal proteins to the cell membrane at their uropods.

Since the identification of CD44 as the principal receptor for the extracellular matrix (ECM) molecule hyaluronan (HA) almost 20 years ago (Aruffo et al., 1990), this protein has puzzled immunologists by virtue of its manifold, at times seemingly contradictory roles. Widely expressed on almost all cells of the body, it serves a broad set of important but frequently redundant functions in organ development, tissue repair, hematopoiesis, and immune defense. Part of the vexing complexity of its biology probably stems from the fact that extensive alternative splicing of the original transcript and diverse posttranslational modifications of the various gene products lead to the generation of many molecular isoforms with varying functions that are differentially expressed in different cell types and at different developmental stages of the same cell type (Ponta et al., 2003).

Naive T cells, like most cells of the immune system in their resting states, express the shortest CD44 splice variant (called CD44s or CD44h), which does not confer binding to HA. It is only upon activation and induction of effector differentiation that T cells express the CD44v5 isoform and that a fraction of them transiently acquire the capability to bind HA via CD44 (Lesley et al., 1994). Experimental data have suggested that these T cells' binding of HA via CD44 impacts their migration to inflamed tissues that are rich in HA by facilitating rolling interactions of blood-borne effector T cells with the postcapillary endothelium, which allows for subsequent extravasation (Degrendele et al., 1997). Such a model of CD44 as an adhesion receptor of effector cells targeted for inflamed tissues is in agreement with the observation that its genetic deficiency confers only a mild immunological phenotype in healthy mice (Schmits et al., 1997) but causes immune dysfunction in several models of infectious and inflammatory disease (Puré and Cuff, 2001). However, many other molecular functions of CD44 have been described, such as signal transduction, coreceptor function, organization of membrane proteins, or binding of matrix metalloproteases for proteolytic activation of cytokines and pericellular ECM degradation. Indeed, a more complex role of CD44 in T cell function has already been heralded, for example, by the finding that it indirectly supports the role of another T cell adhesion receptor, the $\alpha_4\beta_1$ integrin, through the formation of a bimolecular complex via its cytoplasmic domain (Nandi et al., 2004). Now Mrass et al. (2008), by demonstrating that CD44 also allows effector T cells to stabilize polarity independent of its extracellular domain, and at the same time highlighting its optimizing function in the T cell-mediated elimination of tumors, open up another exciting facet of CD44.

Mrass et al. (2008) focus on a mouse model in which the adoptive transfer of in vitro-primed, ovalbumin-specific cytotoxic T lymphocytes (CTLs) facilitates the near-complete rejection of ovalbumin-expressing tumors. CTLs genetically deficient in CD44 initially reject these tumors at the same pace as wild-type CTLs, but they leave behind larger residual tumors and allow for accelerated local recurrence. Upon careful comparative characterization of CTLs lacking CD44, no defects with respect to activation and effector differentiation could be identified.